Diagnostic algorithm for primary immunodeficiency

Professor Elissaveta Naumova

Central laboratory for Clinical immunology
University Hospital Alexandrovska
Sofia, Bulgaria

“Together for integrative approach to rare diseases” – 13-14 June Plovdiv, Bulgaria
Primary immunodeficiency diseases are a heterogeneous group of disorders defined by defects in genes involved in host defense that can be broadly categorized into five groups:

- Primarily antibody deficiencies
- Combined immunodeficiencies
- Other well-defined immunodeficiency syndromes
- Phagocyte disorders
- Complement deficiencies
More than 100 inherited immunodeficiency disorders are currently recognized, and the number continues to increase as more genetic defects are identified. PIDs underlying genetic defects lead to abnormalities in T- and B-cell development at the different stages.
Accurate diagnosis and classification of PID is necessary

WHY?

to decide on appropriate **clinical management**

to enable informed **genetic counseling**

to permit the systematic collection of data on PID through **registries** that will facilitate further study of these rare diseases

to highlight the advances in **gene therapy**
Diagnosis of PID

*How to do it?*

- Medical History
- Physical examination
- Laboratory testing
First step: Screening

- **Antibody mediated immunity**
  - Quantitative immunoglobulines
  - IgG subclasses determination
  - Isohaemagglutinins

- **T-cell immunity**
  - Total lymphocyte count
  - Lateral chest x-ray (infants)
  - Delayed hypersensitivity skin tests (individuals > 2 years of age)

- **Neutrophile**
  - White blood cell count and differential

- **Complement**
  - Total hemolytic complement
  - C3 and C4 concentration
More Detailed Laboratory Tests

- Specific Antibodies (pre/post immunization)
  - Anti-diptheria/tetanus antibodies
  - Anti-pneumococcal polysaccharide antibodies (individuals > 2 years of age)
  - Anti-haemophilus antibodies
More Detailed Laboratory Tests

➢ Cell immunity

• Lymphocyte subset determination

• Mitogen and antigen reactivity

• Enzyme assays (ADA)
More Detailed Laboratory Tests

- Neutrophil function
  - Burst test
  - Chemotaxis
  - Leukocyte phenotypes (CD11a/CD18, CD11b/CD18, CD11c/CD18)

- Complement function
  - Functional assays for complement
<table>
<thead>
<tr>
<th>Phenotyping</th>
<th>Detection of (deficiency of) specific gene product</th>
<th>Functional assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very low B cell numbers</td>
<td>Btk</td>
<td></td>
</tr>
<tr>
<td>T⁻B⁺NK⁻</td>
<td>Common gamma chain</td>
<td></td>
</tr>
<tr>
<td>T⁻B⁺NK⁺</td>
<td>IL7R alpha chain (CD127)</td>
<td></td>
</tr>
<tr>
<td>T⁻B⁻NK⁺⁻</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T⁻B⁻NK⁺</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD8 deficiency</td>
<td>ZAP-70</td>
<td></td>
</tr>
<tr>
<td>HLA class I/ II deficiency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High CD4:CD8 ratio in children</td>
<td>Absence or abnormal expression of CD154 upregulation in vitro CD154 on activated T cells</td>
<td></td>
</tr>
<tr>
<td>Decreased CD3 cells</td>
<td>WASP</td>
<td></td>
</tr>
<tr>
<td>Decreased CD3CD8 cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-2 integrins deficiency (CD18)</td>
<td>CD11b upregulation in vitro</td>
<td></td>
</tr>
<tr>
<td>Cytochrome 558</td>
<td>Oxidative burst</td>
<td></td>
</tr>
<tr>
<td>Immuno phenotype</td>
<td>Defect</td>
<td>Mutated genes</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------------------------------------------------------------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td>T·B⁺NK⁻</td>
<td>Absence of receptors for IL-2, -4, -7, -9, -15 and -21</td>
<td>Common cytokine-receptor γ-chain</td>
</tr>
<tr>
<td></td>
<td>Defect of signaling via IL-2, -4, -7, -9, -15 and -21</td>
<td>JAK3</td>
</tr>
<tr>
<td></td>
<td>Absence of IL-7 receptor α</td>
<td>IL-7 receptor α-chain TCRγ/ε chain</td>
</tr>
<tr>
<td></td>
<td>TCR deficiency</td>
<td></td>
</tr>
<tr>
<td>T·B⁺NK⁺</td>
<td>Defective VDJ recombination</td>
<td>RAG-1 and RAG-2</td>
</tr>
<tr>
<td>T·B⁻NK⁺</td>
<td>Block in purine salvage metabolism</td>
<td>ADA</td>
</tr>
<tr>
<td></td>
<td>Block in purine salvage metabolism</td>
<td>PNP</td>
</tr>
<tr>
<td>T·B⁻NK⁺⁻/⁻</td>
<td>Decreased CD3 cells</td>
<td>Deletion in chr.22q11.2</td>
</tr>
</tbody>
</table>

**Specific (Genetic) Tests**
### Specific (Genetic) Tests

<table>
<thead>
<tr>
<th>Immuno phenotype</th>
<th>Defect</th>
<th>Mutated genes</th>
<th>Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased CD3CD8 cells</td>
<td>ZAP-70 deficiency, WASP deficiency</td>
<td>ZAP-70, WASP</td>
<td>CD8 lymphopenia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>WAS</td>
</tr>
<tr>
<td>Absent/very low B cells</td>
<td>Btk deficiency</td>
<td>Btk, Igµ chain or Igα(CD79a) or the BCLK</td>
<td>X-linked Agammaglobulinemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AR Agammaglobulinemia</td>
</tr>
<tr>
<td>HLA-ClassI -</td>
<td></td>
<td></td>
<td>MHC Class I deficiency</td>
</tr>
<tr>
<td>HLA-ClassII -</td>
<td></td>
<td></td>
<td>MHC Class II deficiency</td>
</tr>
<tr>
<td>IgG and IgA deficiency with normal/elevated IgM</td>
<td>Failure of gene regulation</td>
<td>RFX5, RFXAP, RFXANK, MHC2TA(CIITA)</td>
<td>X-linked Hyper IgM Syndrom</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Non- X-linked Hyper IgM Syndrom</td>
</tr>
</tbody>
</table>
Examples for applying PID diagnostic algorithm in our practice
Primarily humoral (B-cell) immunodeficiencies
X-Linked Agammaglobulinemia

**Definitive diagnostic criteria**

Male patient with less than 2% CD19+ B cells and at least one of the following:
1. Mutation in Btk.
2. Absent Btk mRNA on Northern blot analysis of neutrophils or monocytes.
3. Absent Btk protein in monocytes or platelets.
4. Maternal cousins, uncles, or nephews with less than 2% CD19+ B cells.

IgG, IgA, IgM –not detected by RID

CD3 = 92% 3,068/mm$^3$  
CD19 = 0% 0/mm$^3$  
NK = 8% 250/mm$^3$
Pedigree of family with XLA
Common Variable Immunodeficiency (CVID)

- IgG – 0.5 g/L
- IgA – not detected with RID
- IgM - 0.17 g/L
- CD3 = 78% 6638/mm³
- CD3CD4 = 22% 1872/mm³
- CD3CD8 = 61% 5191/mm³
- CD19 = 16% 1361/mm³
- NK = 2% 170/mm³

**Probable**

Male or female patient who has a marked decrease (at least 2 SD below the mean for age) in serum IgG and IgA and fulfills all of the following criteria:

- onset of immunodeficiency at greater than 2 years of age
- absent isohemagglutinins and/or poor response to vaccines.
- defined causes of hypogammaglobulinemia have been excluded

*Diagnostic criteria PAGID and ESID/Clinical Immunology 1999*
T-cell or combined immunodeficiencies
Severe Combined Immunodeficiency (SCID)

**Definitive diagnostic criteria**

Male or female patient less than 2 years of age with either (a) engraftment of transplacentally acquired maternal T cells or (b) less than 20% CD3+ T cells, an absolute lymphocyte count of less than 3000/mm3, and at least one of the following:

1. Mutation in the cytokine common gamma chain (γc).
3. Mutation in RAG1 or RAG2.
4. Mutation in IL-7Rα.
5. ADA activity of less than 2% of control or mutations in both alleles of ADA.
Specific types of SCID are associated with particular peripheral blood lymphocyte subset abnormalities

- T-B+NK- phenotype
  - mutations in the γc or Jak3 gene
  - mutations in IL-7R α
  - mutations in ADA or in PNP genes

- T-B- NK+ phenotype
  - mutations in the RAG1 or RAG2 genes

- T-B+NK+ phenotype
  - mutations in the γc or Jak3 gene

- T-B-NK- phenotype
  - mutations in the RAG1 or RAG2 genes

T-B+NK- phenotype

mutations in the \( \gamma c \) or Jak3 gene

absence of \( \gamma c \) (CD132) expression

X-Linked Severe Combined Immunodeficiency (XSCID)

Definitive diagnostic criteria

Male patient with either (a) engraftment of transplacentally acquired maternal T cells or (b) less than 10% CD3+ T cells, less than 2% CD16/56+NK cells, and more than 75% CD19+ B cells and who has one of the following:

1. Mutation in the cytokine common gamma chain (γc).
2. Absent γc mRNA on Northern blot analysis of lymphocytes.
3. Absent γc protein on the surface of lymphocytes or lymphocyte cell lines.
4. Maternal cousins, uncles, or nephews with severe combined immunodeficiency.
Bare lymphocyte syndrome

Definitive diagnostic criteria
Male or female patient with decreased intensity of expression (less than 5% of normal) of HLA-DR or DP on B cells or monocyte

- A mutation in one of the following genes: CIITA, RFX-ANK, RFX-5, or RFX-AP.
HLA – DR expression after stimulation

Lack of inducible MHC class II molecules expression

control

patient ASB

NS

PHA

PPD

no proliferative response
The X-linked hyper IgM syndrome (XHIM)

Probable diagnostic criteria

Male patient with serum IgG concentration at least 2SD below normal for age and one of the following:

1. Normal number of T cells and normal T cell proliferation to mitogens.
2. Normal or elevated numbers of B cells but no antigen-specific IgG antibody.
3. One or more of the following infections or complications: recurrent bacterial infections in the first 5 years of life; *Pneumocystis carinii* infection in the first year of life; neutropenia; Cryptosporidium-related diarrhea; Sclerosing cholangitis; Parvovirus-induced aplastic anemia.
4. Absent CD40 ligand cell surface staining on activated CD4+ T cells as assessed by binding to soluble CD40 or by binding of monoclonal antibody to CD40 ligand.
Other well-defined immunodeficiency syndromes
Wiskott–Aldrich Syndrome (WAS)

Definitive diagnostic criteria

Male patient with congenital thrombocytopenia (less than 70,000 platelets/mm³), small platelets and at least one of the following:

1. Mutation in WASP.
2. Absent WASP mRNA on Northern blot analysis of lymphocytes.
3. Absent WASP protein in lymphocytes.
4. Maternal cousins, uncles, or nephews with small platelets and thrombocytopenia.

Phagocyte disorders
Chronic granulomatous disease

**Definitive diagnostic criteria**

Male or female patient with abnormal NBT or respiratory burst in activated neutrophils (less than 5% of control) who has one of the following:

1. Mutation in gp91, p22, p47, or p67 phox.
2. Absent mRNA for one of the above genes by Northern blot analysis.
3. Maternal cousins, uncles, or nephews with an abnormal NBT or respiratory burst.

*Diagnostic criteria PAGID and ESID/Clinical Immunology 1999*
Leukocyte adhesion deficiency

Definitive diagnostic criteria

A male or female patient with decreased intensity of expression of CD18 on neutrophils (less than 5% of normal) and at least one of the following:

1. Mutation in the β2 integrin gene.
2. Absence of β2 integrin mRNA in leukocytes.

*Diagnostic criteria PAGID and ESID/Clinical Immunology 1999*
Main patient registry
>10 000 recorded cases of Primary Immunodeficiencies in Europe